

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 31/20, 31/23, A23K 1/16, 1/18	A1	(11) International Publication Number: WO 98/05319 (43) International Publication Date: 12 February 1998 (12.02.98)
(21) International Application Number: PCT/US97/08716 (22) International Filing Date: 20 May 1997 (20.05.97) (30) Priority Data: 08/693,713 7 August 1996 (07.08.96) US (71) Applicant: WISCONSIN ALUMNI RESEARCH FOUNDATION (US/US); P.O. Box 7365, Madison, WI 53707-7365 (US). (72) Inventors: COOK, Mark, E.; 15 Kewaunee Court, Madison, WI 53705 (US). PARIZA, Michael, W.; 7102 Valhalla Trail, Madison, WI 53719 (US). JEROME, Daria, L.; 5730 Highland Way #204, Middleton, WI 53563 (US). (74) Agent: BAKER, Jean; Quarles & Brady, 411 East Wisconsin Avenue, Milwaukee, WI 53202-4497 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: METHODS OF PREVENTING SKELETAL PROBLEMS IN POULTRY (57) Abstract <p>Methods of treating a bird to prevent skeletal problems consist of administering to the bird a safe and effective amount of CLA (conjugated linoleic acid).</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav	TM	Turkmenistan
BF	Burkina Faso	GR	Greece		Republic of Macedonia	TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

METHODS OF PREVENTING SKELETAL PROBLEMS IN POULTRY

Field of the Invention

The present application generally relates to methods of treating poultry. More particularly, it relates to methods of treating poultry to prevent skeletal problems, especially valgus
5 and varus leg deformities i.e., twisted or bowed legs.

Background of the Invention

Skeletal problems are recognized as one of the four major factors limiting the performance of meat-type birds. The fast-growing broilers and turkeys that reach market weights at
10 earlier ages appear to have more leg problems and suffer greater losses than slower growing birds. There is survey data in the literature that indicates that the body weight of broilers is highly correlated to the severity of leg problems. The leading causes of leg problems in broilers include nutritional
15 disorders, such as rickets; infectious diseases, such as viral arthritis, metabolic conditions, such as tibial dyschondroplasia; conformational problems, such as varus and valgus deformities; and toxins, such as mycotoxins.

Leg problems in broilers can increase mortality and
20 increase the number of culls; they also can increase condemnations from septicemia-toxemia, and increase downgrades from trimming breasts and legs. In addition decreased feed consumption and increased disease also can occur.

It has been estimated that the annual losses due to leg
25 problems in broilers is about \$80 to \$120 million. In turkeys, the cost estimates of annual production losses due to skeletal

-2-

defects in the United States are as high as \$32 million. Leg problems also cause losses for the growers of other birds, such as pheasants, emus, ostriches, rhea and other ratites.

Obviously, it would be advantageous to have methods of treating poultry to prevent skeletal deformities, especially leg deformities.

Brief Summary of the Invention

It is the object of the present invention to disclose a method of preventing skeletal problems in poultry.

10 We have discovered a method of preventing skeletal problems in poultry which comprises administering to a bird susceptible to skeletal problems a safe and effective amount of a conjugated linoleic acid, such as 9,11-octadecadienoic acid and 10,12-octadecadienoic acid, a non-toxic salt of a conjugated linoleic acid, an active ester of a conjugated linoleic acid, active isomers, active metabolites or a mixture thereof.

The conjugated linoleic acids, their non-toxic salts, active esters, active isomers, active metabolites, and mixtures thereof are referred to herein as "CLA".

20 It will be apparent to those skilled in the art that the forementioned objects and other advantages may be achieved by the practice of the present invention.

Description of the Preferred Embodiment

In the preferred method of the present invention, CLA is orally administered to a bird in a safe amount which is effective to prevent skeletal problems, especially leg deformities. Because of the differences in ages, size and

-3-

nature of birds, the amounts which are safe and effective may vary considerably. Since CLA is a natural food ingredient and it is relatively non-toxic, the amounts which can be administered in the methods of the invention are not critical as long as they are enough to be effective.

The practice of the present invention is further illustrated by the examples which follow:

Example 1

Six pens of 5 chicks were fed CLA at 0, 0.1875, 0.375, and 0.75% of the diet. At 3 weeks of age the legs of the chicks were scored for twisted, bowed condition. Scoring was 1=normal, 2=slight, 3=moderate, and 4=severe (essentially crippled). The results after 3 weeks are shown in Table 1.

Table 1

CLA Content	Body Weight (g)	Mean Leg Score Mean \pm Std Error	Percent with Severe bending
0%	605	2.0 \pm 0.2	27 \pm 7*
0.1875%	606	1.7 \pm 0.2	13 \pm 10
0.375%	618	1.6 \pm 0.4	7 \pm 4
0.75%	601	1.4 \pm 0.2	3 \pm 3*

Table 2 shows the results obtained after 8 weeks in control birds and the 10 birds fed the diet containing 0.75% CLA.

Table 2

CLA Content	Body Weight (g)	Mean Leg Score Mean \pm Std Error	Percent with Severe bending
0%	2372	2.2	33
0.75%	2745	1.1	0

*Only 1 of 30 chicks had severe score in high CLA, with 8 out of 30 in control.

-4-

After 9 weeks the control birds had an average body weight of 2684g and the birds receiving CLA had an average body weight of 3220g.

As can be seen from the above, the birds fed the 0.75% CLA diet gained significantly more weight than the control birds. Similar results can be obtained in turkeys, ducks, geese, emus, pheasants, ostriches, rheas and other ratites.

The method of the present invention may take several embodiments. In one embodiment, the CLA is added to a bird's diet by adding the CLA to poultry feed. In another embodiment, the CLA can be administered to the bird in a veterinary composition containing a safe and effective dose of the CLA. In still another embodiment, the bird is fed a food product, such as milk or egg solids, which have been enriched so that they contain high concentrations of CLA.

The poultry feed and veterinary preparations for use in the methods of the present invention preferably are those containing the CLA in the form of the triglyceride esters in combination with a conventional poultry feed or approved veterinary diluent. Other active forms of CLA including, without limitation, the non-toxic salts and mixtures can be used.

The free conjugated linoleic acids (CLA) have been previously isolated from fried meats and described as anticarcinogens by Y. L. Ha, N. K. Grimm and M. W. Pariza, in Carcinogenesis Vol. 8, No. 12, pp. 1881-1887 (1987). Since then, they have been found in some processed cheese products (Y.L. Ha, N. K. Grimm and M. W. Pariza, in J. Agric. Food Chem., Vol. 37, No. 1, pp. 75-81 (1987)). The free acid forms of the CLA may be prepared by isomerizing linoleic acid. The non-

-5-


toxic salts of the free CLA acids may be made by reacting the free acids with a non-toxic base. Natural CLA may also be prepared from linoleic acid by the action of W¹²-cis, W¹¹-transisomerase from a harmless microorganism such as the Rumen
5 bacterium Butyrivibrio fibrisolvens. Harmless microorganisms in the intestinal tracts of rats and other monogastric animals may also convert linoleic acid to CLA (S.F. Chin, W. Liu, K. Albright and M.W. Pariza, 1992, FASEB J.6:Abstract #2665).

The CLA obtained by the practice of the described methods
10 of preparation contains one or more of the 9,11-octadecadienoic acids and/or 10,12-octadecadienoic acids and active isomers thereof. It may be free or bound chemically through ester linkages. The CLA is heat stable and can be used as is, or dried and powdered. The free acids are readily converted into
15 non-toxic salts, such as the sodium or potassium salts, by reacting chemically equivalent amounts of the free acid with an alkali hydroxide at a pH of about 8 to 9.

Theoretically, 8 possible geometric isomers of 9,11- and 10,12-octadecadienoic acid (c9,c11; c9,t11; t9,c11; t9,t11;
20 c10,c12; c10,t12; t10,c12 and t10,t12) would form from the isomerization of c9,c12-octadecadienoic acid. As a result of the isomerization, only four isomers (c9,c11; c9,t11; t10,c12; and c10,c12) would be expected. However, of the four isomers, c9,t11- and t10,c12- isomers are predominantly produced during
25 the autoxidation or alkali-isomerization of c9,c12-linoleic acid due to the co-planar characteristics of 5 carbon atoms around a conjugated double-bond and spatial conflict of the resonance radical. The remaining two c,c-isomers are minor contributors.

The relatively higher distribution of the t,t-isomers of 9,11- or 10,12-octadecadienoic acid apparently results from the further stabilization of c9,t11- or t10,c12-geometric isomers, which is thermodynamically preferred, during an extended
5 processing time or long aging period. Additionally the t,t-isomer of 9,11- or 10,12-octadecadienoic acid that was predominantly formed during the isomerization of linoleic acid geometrical isomers (t9,t12-, c9,t12- and t9,c12-octadecadienoic acid) may influence the final ratio of the isomers or the final
10 CLA content in the samples.

Linoleic acid geometrical isomers also influence the distribution of minor contributors (c,c-isomers of 9,11- and 10,12-, t9,c11- and c11,t12-octadecadienoic acids). The 11,13-isomer might be produced as a minor product from c9,c12-
15 octadecadienoic acid or from its isomeric forms during processing.



The CLA can be administered in the form of veterinary compositions, such as solutions or emulsions. The exact amount to be administered, of course, depends upon the form of CLA
20 employed, and the route of administration. Generally, the amount employed of the non-toxic salts in a veterinary composition will range from about one part per million (ppm) to about 10,000 ppm of CLA of the bird's diet. The preferred veterinary compositions of CLA contain the non-toxic sodium or
25 potassium salt of CLA in combination with a suitable diluent. In addition to solutions or suspensions intended for oral administration the product can be a powder or a crushable tablet. When the compositions are solutions or suspensions

-7-

intended for parenteral administration the preferred diluent will be Sterile Water for Injection U.S.P.

The amounts of CLA to be added to poultry feed can range from about .01% to about 5.0% or more by weight of the bird's
5 diet. The bird can be fed a diet containing the CLA from birth to harvesting or for some other period until at least some of beneficial effects of the CLA are obtained.

It will be readily apparent to those skilled in the art that a number of modifications or changes may be made without
10 departing from the spirit and scope of the present invention. Therefore, the invention is only to be limited by the claims.

We claim:

1. A method of preventing skeletal problems in a bird, said method comprising administering to said bird a safe amount of CLA which is effective to prevent skeletal problems.
- 5 2. The method of claim 1 in which the CLA is a non-toxic salt of a conjugated linoleic acid.
3. The method of claim 1 in which the CLA is a triglyceride of a conjugated linoleic acid.
4. The method of claim 1 in which the bird is a chicken.
- 10 5. The method of claim 1 in which the bird is a turkey.
6. The method of claim 1 in which the bird is a duck.
7. The method of claim 1 in which the CLA is administered orally.
8. The method of claim 1 in which the CLA is administered
15 parenterally.
9. The method of claim 1 in which the skeletal problems are leg deformities.

10. A method of improving growth and feed efficiency in birds and reducing losses due to culls, condemnation and mortality which comprises administering to said birds a safe amount of CLA which is effective to prevent leg deformities and
5 other skeletal problems.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/08716

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/20 A61K31/23 A23K1/16 A23K1/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 16690 A (WISCONSIN ALUMNI RES FOUND) 4 August 1994 see page 1, line 3 - page 2, line 27 see page 11, line 10 - page 14, line 16 see examples 1-3,7 see claims 1,2,4-6 ---	1-4,7,8, 10
A	WO 96 06605 A (WISCONSIN ALUMNI RES FOUND) 7 March 1996 see page 2, line 2 - page 3, line 7 see claims 1-5,7,10 --- -/-	1-3,7,8, 10

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- * "A" document defining the general state of the art which is not considered to be of particular relevance
- * "E" earlier document but published on or after the international filing date
- * "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- * "O" document referring to an oral disclosure, use, exhibition or other means
- * "P" document published prior to the international filing date but later than the priority date claimed

* "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

* "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

* "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

* "A" document member of the same patent family

Date of the actual completion of the international search

24 September 1997

Date of mailing of the international search report

07.10.97

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+ 31-70) 340-3016

Authorized officer

Dekeirel, M

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/08716

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	M.PARIZA ET AL.: "Mechanism of body fat reduction by conjugated linoleic acid" FASEB JOURNAL., vol. 11, no. 3, 1997, FOR EXPERIMENTAL BIOLOGY, BETHESDA, MD US, page A139 XP002041644 see page A139, column 2, Abstract 813 ---	1-4,10
A	EP 0 579 901 A (WISCONSIN ALUMNI RES FOUND) 26 January 1994 see page 2, line 13 - page 3, line 4 see examples 1,2 see claims 1-14 ---	1-4,7,8, 10
P,A	Y.LI ET AL.: "Dietary conjugated linoleic acid reduced ex vivo bone PGE2 production in rats" FASEB JOURNAL., vol. 11, no. 3, 1997, FOR EXPERIMENTAL BIOLOGY, BETHESDA, MD US, page A165 XP002041645 see page A165, column 1, Abstract 962 ---	1,10
P,A	WO 96 34855 A (SCOTIA HOLDINGS PLC ;HORROBIN DAVID F (GB); MANKU MEHAR (GB); MCMO) 7 November 1996 see claims 1,6,9,10 -----	1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/08716

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 1-10 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/08716

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9416690 A	04-08-94	US 5428072 A	27-06-95
		EP 0680318 A	08-11-95
		JP 8505775 T	25-06-96
		US 5504114 A	02-04-96
		US 5554646 A	10-09-96

WO 9606605 A	07-03-96	US 5554646 A	10-09-96
		EP 0731699 A	18-09-96

EP 0579901 A	26-01-94	US 5430066 A	04-07-95
		DE 69301693 D	11-04-96
		DE 69301693 T	25-07-96
		US 5428072 A	27-06-95
		US 5504114 A	02-04-96

WO 9634855 A	07-11-96	AU 5507996 A	21-11-96
		AU 5508096 A	21-11-96
		AU 5508196 A	21-11-96
		WO 9634846 A	07-11-96
		WO 9634858 A	07-11-96
